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Phytoscreening and phytoextraction of heavy metals at Danish polluted sites using willow and poplar trees

Mette Algreen · Stefan Trapp · Arno Rein

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Abstract The main purpose of this study was to determine typical concentrations of heavy metals (HM) in wood from willows and poplars, in order to test the feasibility of phytoscreening and phytoextraction of HM. Samples were taken from one strongly, one moderately, and one slightly polluted site and from three reference sites. Wood from both tree species had similar background concentrations at 0.5 mg kg^{-1} for cadmium (Cd), 1.6 mg kg^{-1} for copper (Cu), 0.3 mg kg^{-1} for nickel (Ni), and 25 mg kg^{-1} for zinc (Zn). Concentrations of chromium (Cr) and lead (Pb) were below or close to detection limit. Concentrations in wood from the highly polluted site were significantly elevated, compared to references, in particular for willow. The conclusion from these results is that tree coring could be used successfully to identify strongly heavy metal-polluted soil for Cd, Cu, Ni, Zn, and that willow trees were superior to poplars, except when screening for Ni. Phytoextraction of HMs was quantified from measured concentration in wood at the most polluted site. Extraction efficiencies were best for willows and Cd, but below 0.5 % over 10 years, and below 1‰ in 10 years for all other HMs.

Keywords Extraction efficiencies · Phytoremediation · Phytotechnologies · Plant uptake · Soil contamination · Toxic elements · Tree core sampling · Wood

Background, aim, and scope

The examination of subsurface pollution by analyzing tree cores was started in the 1990s in the USA (Schumacher et al. 2004; Vroblesky et al. 1999). Today, phytoscreening of soil and groundwater has become a scientifically validated and recognized method (Sorek et al. 2008; Gopalakrishnan et al. 2007) and has frequently been used to investigate plumes of chlorinated solvents, such as tetrachloroethene, trichloroethene, and cis-1,2-dichloroethene (Sorek et al. 2008; Larsen et al. 2008). The principle underlying the method is that contaminants are taken up by roots and translocated upwards to the stem. By use of a borer, tree cores can be taken from the trunks of trees for chemical analysis. The presence of subsurface pollutants in the wood was found to indicate the presence of these pollutants in soil and/or groundwater (Burken et al. 2011).

Using tree core sampling as a phytoscreening technique for chlorinated solvents is now an established technique (Holm et al. 2011; Vroblesky 2008; Trapp et al. 2007) with beginning commercial use. Recent developments include applying solid-phase microextraction methods for in planta quantification of chlorinated compounds (Sheehan et al. 2012). However, what works so well for volatile organic compounds seems to provide more difficulties for heavy metals (HM). Algreen et al. (2012) tested tree coring of toxic elements and HMs for an abandoned waste site in Norway by comparing concentrations in wood samples from the test site with concentrations in samples from reference sites. In most cases, the concentrations of the investigated elements were higher in wood from the test site. But the authors also found a high variation in heavy metal content of

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the wood, and a dependency on tree species. This may be due to the fact that several heavy metals are essential for plant growth (McLaughlin et al. 2011). The mere occurrence of HM in wood does, therefore, not indicate elevated levels or pollution of subsurface. Willows (*Salix* sp.) and poplars (*Populus* sp.) usually had the highest contents of HM in wood, compared to other tree species, and were preferably sampled. However, the observed differences between contaminated test site and (nominally) unpolluted reference site were not always statistically significant. A main conclusion from that study was that phytoscreening with tree cores is more difficult to use for HM than for chlorinated solvents. Overall, tree coring seemed a promising way to find subsurface contamination, but the results of the study did not allow a final judgment of the feasibility of this method for HM (Algreen et al. 2012).

Numerous studies deal with the uptake of HM into vegetation (McLaughlin et al. 2011). For example, the BAPPET database (2008) reports measured concentrations of metals in plant tissue and soil, with many thousand entries. Most crops and vegetables are considered, but there are no data on wood. Generally, only few scientific studies focused on HM concentrations in wood (Algreen et al. 2012). This is quite surprising, giving the high interest in phytoextraction of metals. Rock (2003) gives an overview of 33 field trials for phytoremediation, and trees such as willow (*Salix* sp.) and hybrid poplar (*Populus* sp.) are among the most frequently used species. However, in most of these field studies, the effect of phytoextraction on metal concentrations in soil was negligible or insignificant. Elevated heavy metal concentrations in vegetation have also been used for prospecting ore deposits (Hohl 1981).

We applied the tree coring method and took wood samples from willows (*Salix* sp.) and poplars (*Populus* sp.) at three polluted test sites and three non-polluted reference sites in Denmark. The concentrations of cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) in wood and soil were analyzed and evaluated. Goals of this study were to determine typical concentrations of HM in wood from willows and poplars and to investigate tree core sampling as a low invasive screening method. The latter included testing for differences of HM concentrations in wood from trees growing under background conditions and on polluted sites, testing for differences in HM uptake between the two tree species, and studying the relation between HM in soil and wood. Another goal was to evaluate the feasibility of HM extraction from contaminated sites by performing a mass balance, based on a 10-year field trial.

Materials and methods

Test sites

Three test sites located in Denmark were selected for tree core and soil sampling (Fig. S1 in the Supporting Information).

Test site 1, Valby Most samples were taken from the former sludge disposal site in Valby. The site is distant from traffic and close to the Baltic Sea. The site has a known high pollution with organic and inorganic pollutants and was from 1999 to 2005 used as phytoremediation test site by the Danish Environmental Protection Agency (Oberender et al. 2009). Willows (*Salix viminalis* varieties) and poplars (mostly *Populus trichocarpa*) were planted in 1999 at the site in two separate areas (Fig. S1a). Approximately 15 % of the trees died the first year, and additional trees were planted in 2000. Remaining trees have shown good growth, but some trees had curled and spotted leaves indicating stress and/or diseases (Oberender et al. 2009). The concentrations of Cd, Cr, Cu, Pb, Ni, and Zn (among others) in the soil are high (up to 30, 1,300, 700, 200, 4,400, and 6,200 mg kg⁻¹ total soil dry weight, respectively), classifying the soil as strongly polluted. Samples from trees growing in the arboretum in Hørsholm, North Sealand, Denmark, were taken as reference.

Test site 2, Frederiksværk A second sampling site is a steel work in Frederiksværk (Fig. S1b). Hazardous waste was deposited at the site from 1942 to the late 1980s. The waste consisted of steel scrap and dross from the production, but also chemicals used in the production. The major pollutants at the site were Cd, Cr, Cu, Pb, and Zn. The zones with highest contamination were remediated and are without trees. A site with lower HM content, labeled as “potentially polluted zone” was used for this study. Further information about the site is not available. The reference site was a presumably unpolluted area nearby.

Test site 3, Hillerød Between 1936 and 1976, this site was used as wood proofing facility (Fig. S1c). This resulted in a heterogeneous soil contamination with arsenic, chromium, and copper. The site has not been remediated (further details are reported by Skov og Naturstyrelsen 1989a, b). Reference samples were taken nearby, from an area assumed to be unpolluted.

Sampling

Tree cores Sampling was done according to the Guide to Phytoscreening (Holm et al. 2011). Tree core samples were taken at a height of 1 m using a 6 mm increment borer (Suunto, Finland). Three tree cores with a length of 6 cm were taken around the tree. The outer centimeter, containing the bark and the phloem, was discarded to avoid atmospheric influence. The tree cores from each tree were mixed together in plastic bags for storage until sample preparation. Tree core sampling from test site 1 was performed first in June and September 2011, and 18 samples from willows (*S. viminalis*) and 18 from poplars (*P. trichocarpa*) were taken. From the reference site, Arboretum in Hørsholm, four willows (*Salix*

sp.), and four poplars (*Populus* sp.) were sampled late September 2011. At test site 2, 18 poplars (*Populus nigra*) arranged in a line, as well as two poplars from the nearby reference site were sampled in spring 2010. At test site 3, nine willows (*Salix* sp.) placed at three locations and two willows from the reference site were sampled in summer 2010.

Soil samples The content of HM in soil was analyzed in soil samples from each test site. Each soil sample consisted of three soil cores down to 0.7-m depth around the crown of the tree. The upper 20 cm (top soil) were removed and the remaining soil was mixed together to one sample. The samples were stored in plastic bags until preparation for analysis. The number of samples varied between sites, depending on the site conditions (presence and location of trees, accessibility for soil sampling). From test site 1, three samples from the area of willows and five samples from the area of poplars were taken in the beginning of June 2011. From test site 2, eight soil samples (from every second tree) and from test site 3 three soil samples (one from each area) were sampled additionally with the tree core samples. One soil sample from the reference sites were also taken. Sampling at test site 1 in 2001 is described by Oberender et al. (2009).

Sample preparation and analytical method

Tree core samples Tree core samples were divided into smaller pieces and dried at 75 °C for approximately 24 h to constant weight. Between 0.5 to 0.8 g of the dried sample were weighed into a 50-ml volumetric flask (in duplicates). Ten milliliter 65 % HNO₃ was added, and the flask was placed on a sand bath at 70–80 °C or until the wood was dissolved (approximately 2 h). Flasks were then removed and cooled at room temperature for 10 min before 2.5 ml 30 % H₂O₂ was added. The flasks were placed back on the sand bath until the gas reaction was completed. The procedure was repeated with additional 2.5 ml 30 % H₂O₂. Milli-Q water was added to a volume of 50 ml, and the flask was shaken. Approximately 3 ml of the sample was transferred to a centrifuge glass, the glass was shaken and emptied. The rest of the sample was transferred to the same centrifuge glass and centrifuged for 10 min with 2,500 rpm. The supernatant was transferred to plastic (PE) bottles for storage at room temperature. Seven milliliter of sample was transferred to the test tube for analysis.

Soil samples Soil samples were dried at approximately 23 °C for 3 days to constant weight. For measurements of the total concentration, further sample preparation was done analogue to the sample preparation of the tree core samples. For measurement of the easily extractable concentration of HM, approximately 8 g soil was weighed into blue cap bottles (in

duplicates) and 90 ml 1 mM CaCl₂ was added. The bottles were placed on a shaking bench for 24 h. After shaking, approximately 3 ml of sample were transferred to a centrifuge glass, shaken, and emptied. The rest of the sample was transferred to the same centrifuge glass and centrifuged for 10 min with 2,500 rpm. The supernatant was transferred to plastic (PE) bottles for storage at room temperature. Seven milliliter of sample was transferred to test tubes for analysis. Preparation and analysis of soil samples taken in 2001 at test site 1 are described in Oberender et al. (2009).

All equipment used for sample preparation was acid washed before use. All soil samples were prepared and analyzed after working with the tree core samples to avoid contamination of the tree core samples from the soil samples.

Analytical method All analyses were performed on inductively coupled plasma optical emission spectrometry (Varian, Vista-MPX) and with an internal standard (Yttrium 1 mg/L/HNO₃ 1 % w/w). The calibration curves were done from nine standards in the concentration interval of 0.3–1,012 µg metal/L. Samples measured above 1,012 µg metal/L were diluted and reanalyzed. Six metals were analyzed as follows: Cd (228.802 nm λ), Cr (205.560 nm λ), Cu (327.395 nm λ), Ni (231.604 nm λ), Pb (220.353 nm λ), and Zn (206.200 nm λ). All concentrations in soil and wood are given for the dry weight.

Quality control The method of sample preparation was validated by comparison to the referenced soil standard QC loam soil (Sigma-Aldrich, DK). The analytical method was validated by spike samples, controls, method blank samples, and total blank samples. Measurements below detection limit (DL) were adjusted to ½ of DL (data for DL are shown in Table S1, Supporting Information).

Calculations

Statistical tests The normal distribution of the Valby data was tested by a Kolmogorov–Smirnov test at $\alpha=5\%$, and normal distribution could not be rejected in any case. Therefore, parametrical statistical tests were used. A two-tailed *t* test with error probability of 0.05 ($\alpha=5\%$) was applied to test for significance of differences between mean concentration in wood and soil from test and reference sites, and from willows and poplars. The relation between concentrations of HM in wood and soil (total and easily extractable concentration) was evaluated by Pearson linear correlation and regression analysis.

Bioconcentration factor in trees The bioconcentration factor (BCF) was calculated from the concentration of metals measured in wood C_{Wood} (in milligrams per kilogram) divided by

the total metal concentration measured in the soil C_{Soil} (in milligrams per kilogram) as follows:

$$BCF = C_{Wood} / C_{Soil, total} \quad (1)$$

The BCF values were derived as the average of each sample pair (wood vs. soil) at each site and for each tree species. At test sites 2 and 3, more than one tree core sample was related to one soil sample, and average concentrations in wood were used for the calculation.

Alternatively, the BCF was derived from the slope of the regression between C_{Wood} and C_{Soil} (Trapp and Legind 2011). The advantage is that both low and high concentrations contribute. A second advantage is that the Y-intercept of the regression can be interpreted as background concentration of HM in wood which is independent of the concentration in soil. The strength of the relation between C_{Wood} and C_{Soil} can be judged from the coefficient of determination R^2 , which describes the variance in Y (concentration in wood) that is explained by the regression to concentration in soil. The slope is significant when $R^2 > R^2_{crit}$ (Sachs 1992).

Mass balance calculations Metal concentrations in soil from test site 1 were obtained in 2001 and 2011. Additionally, the removal of HM from soil due to phytoextraction by trees can be calculated as follows:

$$\Delta m = -\Delta M_{Wood} \times C_{Wood} \quad (2)$$

Where Δm (in milligrams) is the mass of HM extracted from soil, C_{Wood} is the measured concentration of HM in wood and ΔM_{Wood} is the mass of wood grown in the 10 years since planting (approximately 10 kg m^{-2} for willows and 15 kg m^{-2} for poplar (BSELF 2012)). These typical values do not consider possible growth reductions due to toxic effects by soil contamination. The resulting change in concentration depends on the soil volume assumed for extraction. With a depth of 0.7 m (maximum depth of soil samples) and a typical soil dry density of 1.3 kg L^{-1} , the soil mass M_{Soil} is 910 kg. The calculated change of concentration in soil over the 10 years ΔC (in milligrams per kilogram) is as follows:

$$\Delta C_{Soil} = \Delta m / M_{Soil} \quad (3)$$

Calculations of the time span t (in year) required to reach the legal standards by phytoextraction were done assuming constant wood growth and BCF. Based upon the equations above, the differential equation for the change of concentration in soil is obtained as follows:

$$\frac{dC_{Soil}}{dt} = -BCF \times \frac{dM_{Wood}}{dt \times M_{Soil}} \times C_{Soil} = -k \times C_{Soil} \quad (4)$$

Where dM_{Wood}/dt (in kilograms per year) is the change of wood mass with time. With a constant extraction rate coefficient k (per year), it follows from the analytical solution that the time t to reach $C_{Soil}(t)$ (here: legal standard) is as follows:

$$t = \frac{(\ln(C_{Soil}(0)/C_{Soil}(t)))}{k} \quad (5)$$

Results

Concentrations in soil The measured total and easily extractable concentrations of HM in soil from the test and reference sites are shown in Table 1. Average and range (minimum to maximum, using all duplicates) are given there, and Table S2 (Supporting Information) provides also standard deviation, median, minimum, and maximum. All concentrations of HM in total soil were above DL, but results for the easily extractable concentration were often below. For all sites, median and average are similar, indicating symmetrical distribution of data (Table S2). Generally, concentrations were highest for Zn and lowest for Cd. There is large difference between the sites, with test site 1 having by far the highest concentrations of all metals, followed by test site 2. Test site 3 and the reference sites have similar, and low, level of pollution in soil. Thus, tree core sampling results from test site 1 are of most relevance for the test of tree coring as a screening method.

Concentrations in tree cores Concentrations measured in the tree core samples are summarized in Table 1 (for more details see Table S3, Supporting Information). The average and range of metal concentrations in willows and poplars can also be seen in Fig. 1. The concentrations of Cd, Cu, Ni, and Zn in wood were in all, except a few cases, above DL. For Cr and Pb, almost all concentrations in wood were below DL and are not shown and not evaluated further. Willows showed generally higher concentrations of Cd, Cu, and Zn than poplars. Only for Ni, poplars had the higher concentration in wood.

Significant differences in concentrations from test and reference sites The statistical significance of differences between results from test and reference site was tested both for concentrations in soil and in wood (Table S4, Supporting Information). The total concentrations in soil of test site 1 and 2 were significantly higher than in their reference sites. Contrary, test site 3 had significantly lower levels of HM than reference site 3, except for Cu. For the easily extractable concentrations in soil, the difference was significant for all HM on test and reference site 1. For test and reference site 2, the difference in easy extractable HM was also significant except for Cd. There was no significant difference for test and reference site 3. For wood, the differences in concentration from test and reference site 1 were all significant, except Cu in poplar wood. At test site 2, no

Table 1 Total and easily extractable concentrations of HM in soil and HM concentration in wood (average values, ranges given in brackets)

| | C _{Soil,total} (mg kg ⁻¹) | C _{Soil,extractable} (mg kg ⁻¹) | C _{Wood} (mg kg ⁻¹) | C _{Soil,total} (mg kg ⁻¹) | C _{Soil,extractable} (mg kg ⁻¹) | C _{Wood} (mg kg ⁻¹) |
|----|------------------------------------------------|------------------------------------------------------|------------------------------------------|------------------------------------------------|------------------------------------------------------|------------------------------------------|
| | Test site 1, willow area | | | Reference site 1, willow area | | |
| | 3 soil samples, <i>n</i> =6 | | 18 trees, <i>n</i> =36 | 3 soil samples, <i>n</i> =6 | | 4 trees, <i>n</i> =8 |
| Cd | 23.9 (16.8–33.9) | 0.014 (0.005–0.027) | 3.08 (0.84–4.75) | 0.23 (0.21–0.26) | 0.0036 (0.0022–0.0055) | 0.65 (0.24–1.6) |
| Cu | 588 (443–708) | 1.98 (1.38–2.67) | 10.7 (1.96–33.4) | 4.36 (3.93–5.16) | 0.025 (0.013–0.029) | 1.68 (1.3–2.59) |
| Ni | 132 (89.3–166) | 0.97 (0.64–1.23) | 0.48 (0.28–1.24) | 4.51 (4.27–4.96) | 0.026 (0.012–0.044) | 0.095 (0.081–0.12) |
| Zn | 3,270 (2,236–4,403) | 2.20 (1.28–4.33) | 106 (55.1–188) | 26.7 (22.6–31.5) | 0.10 (0.046–0.19) | 21.6 (13–31.4) |
| | Test site 1, poplar area | | | Reference site 1, poplar area | | |
| | 5 soil samples, <i>n</i> =10 | | 18 trees, <i>n</i> =36 | 3 soil samples, <i>n</i> =6 | | 4 trees, <i>n</i> =8 |
| Cd | 10.9 (8.64–14.5) | 0.012 (0.0071–0.016) | 0.62 (0.36–1.17) | 0.42 (0.11–1.06) | 0.0022 (0.0022–0.0023) | 0.52 (0.16–1.17) |
| Cu | 651 (575–736) | 2.57 (2.08–3.01) | 1.21 (0.69–2.82) | 11.7 (1.53–29.4) | 0.033 (0.013–0.058) | 1.11 (0.57–2.95) |
| Ni | 153 (104–212) | 1.89 (1.2–2.32) | 0.74 (0.41–1.97) | 7.37 (3.48–16.6) | 0.043 (0.035–0.056) | 0.49 (0.081–0.85) |
| Zn | 2,964 (2,481–3,500) | 3.82 (2.57–4.94) | 32.9 (19.8–76.1) | 20.9 (11.1–44.6) | 0.072 (0.047–0.12) | 25.3 (6.54–76.1) |
| | Test site 2 (poplars) | | | Reference site 2 (poplars) | | |
| | 8 soil samples, <i>n</i> =16 | | 18 trees, <i>n</i> =36 | 1 soil sample, <i>n</i> =2 | | 2 trees, <i>n</i> =4 |
| Cd | 0.99 (0.29–3.45) | 0.0057 (0.0015–0.011) | 0.42 (0.21–0.67) | 0.43 (0.31–0.54) | 0.0025 (0.0015–0.0036) | 0.52 (0.28–0.74) |
| Cu | 67.4 (9–169) | 0.43 (0.11–0.91) | 1.64 (0.99–3.03) | 8.34 (7.82–8.85) | 0.11 (0.11–0.11) | 1.20 (1.09–1.35) |
| Ni | 15.8 (6.25–36.3) | 0.077 (0.011–0.2) | 0.34 (0.12–0.75) | 6.11 (4.72–7.51) | 0.014 (0.011–0.018) | 0.27 (0.19–0.34) |
| Zn | 243 (36.6–645) | 1.29 (0.34–2.9) | 35.7 (16.7–70.1) | 55.6 (41.5–69.7) | 0.17 (0.094–0.25) | 20.6 (15.2–28.3) |
| | Test site 3 (willows) | | | Reference site 3 (willows) | | |
| | 3 soil samples, <i>n</i> =6 | | 9 trees, <i>n</i> =17 | 1 soil sample, <i>n</i> =2 | | 2 trees, <i>n</i> =4 |
| Cd | 0.16 (0.13–0.19) | 0.068 (0.015–0.18) | 0.22 (0.07–0.46) | 0.31 (0.29–0.33) | 0.115 (0.104–0.125) | 0.86 (0.75–1.04) |
| Cu | 8.51 (4.19–12.4) | 0.73 (0.45–1.32) | 1.51 (0.9–2.86) | 6.39 (6.2–6.59) | 0.45 (0.45–0.45) | 1.79 (1.26–2.34) |
| Ni | 3.46 (2.09–4.69) | 0.27 (0.039–0.79) | 0.36 (0.12–0.69) | 9.18 (8.89–9.47) | 0.43 (0.36–0.49) | 0.51 (0.33–0.72) |
| Zn | 24.4 (20–27.2) | 4.44 (0.25–12) | 19.6 (10.8–30) | 30.9 (29.8–32) | 5.30 (4.99–5.6) | 24.1 (20–28.9) |

significant differences were found, and at test site 3, only the concentration of Cd in willows differed significantly, which again was higher in the samples from reference site 3.

Difference between tree species At a significance level of $\alpha=5\%$, willows show significantly higher concentrations of Cd, Cu, and Zn and poplar of Ni at test site 1. The same result is obtained if concentrations in tree species from all sites together are tested for species differences. For reference site 1, poplar wood has significantly higher concentrations of Zn than willow wood (Table S5, Supporting Information).

Relation between concentration in wood and in soil Concentrations in wood versus concentrations in soil are shown in Figs. 2 (total concentration) and S2 (easily extractable concentration in soil). Measured concentrations in wood are highest for $\text{Zn} > \text{Cu} > \text{Cd} > \text{Ni}$ for both tree species. Except for Cu and Zn in poplar wood, concentrations in wood increase with increasing soil concentrations, and generally more in willow wood except for Ni. Table 2 shows the BCF values derived from the slopes of the linear regression between all concentrations in wood and soil, and the Y-intercept interpreted as background concentration in wood. Two of the eight linear regressions have an explained variance (R^2) above 70 %; four regression coefficients are

significant at an error probability of 5 %. The highest slope of the regression line between concentrations in wood and in soil was obtained for Cd, both for willows and poplars. Regressions were also made for the relation of concentration in wood to the easily extractable concentration in soil (Fig. S2, Supporting Information). Only in two (out of eight) cases, R^2 improves (Cu in willows, Ni in poplars, not shown). BCF values derived with Eq. (1) from average concentrations in wood and soils at each site are only useful for test site 1. For all other sites, measured concentrations in wood are close to background. This can be seen by comparison of the Y-intercept of the BCF regressions (Table 2) with the mean concentrations in willow wood of the sites (Table 1), which are rather similar for all HM and the reference sites as well as test sites 2 and 3. Overall, willows take HM better up, and concentrations in wood show a stronger relation to those in soil. Consequently, willows are better indicators of subsurface HM than poplars.

Discussion

Level of soil contamination The usual background level in Danish soils is for Cd from 0.003 to 0.5 mg kg^{-1} , for Cu at

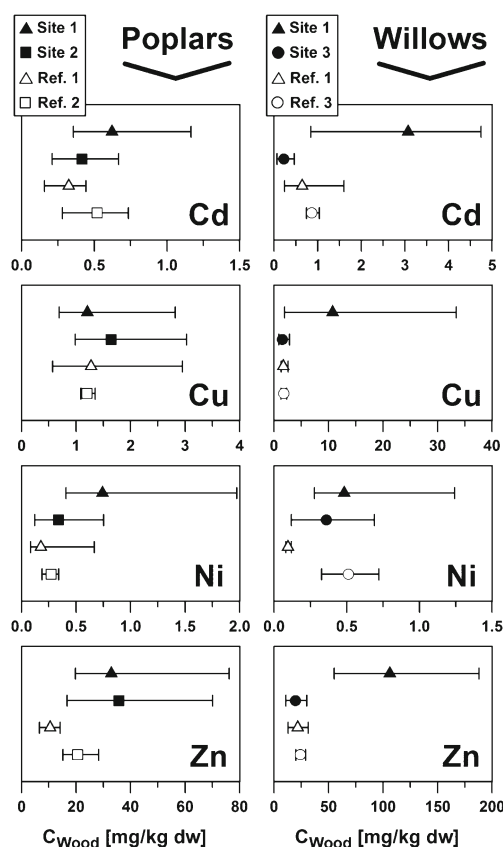


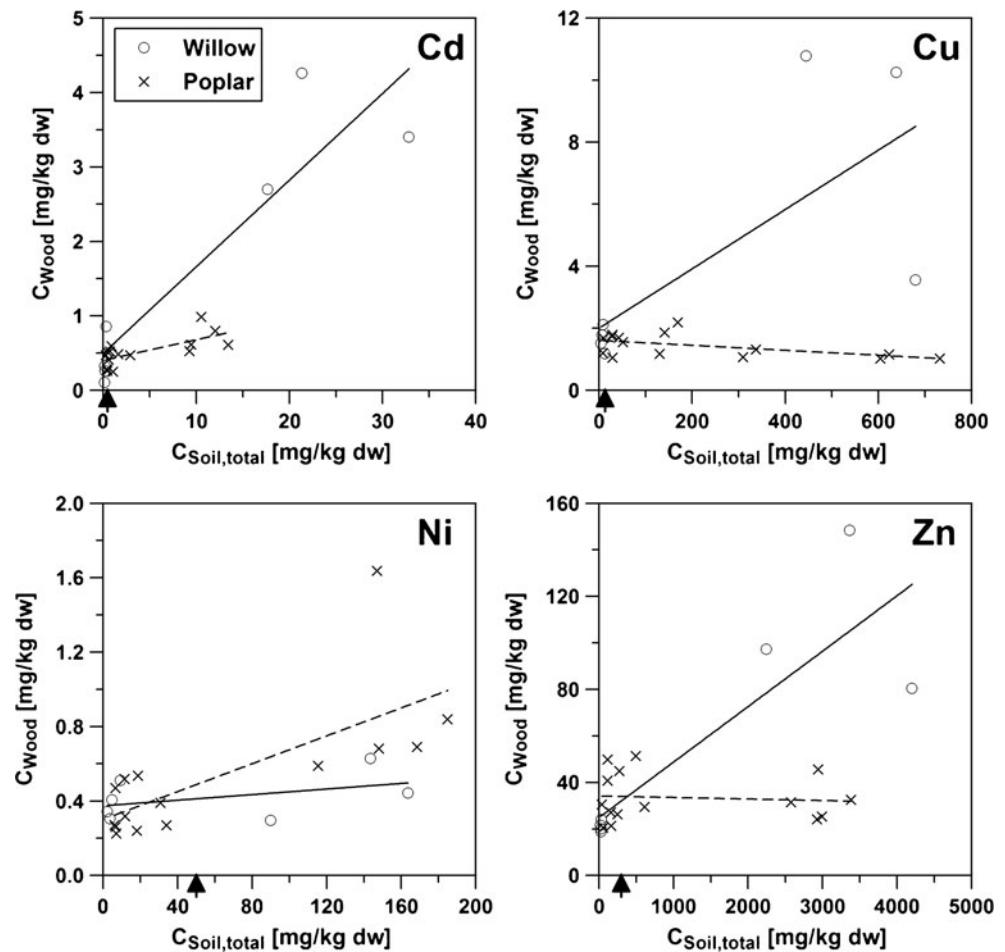
Fig. 1 HM concentrations measured in the wood of poplars and willows from strongly polluted test site 1, slightly polluted test site 2, and non-polluted test site 3 and reference sites (Ref. 1 to 3). Average concentrations, error bars indicate range

about 13 mg kg^{-1} , for Ni from 0.1 to 50 mg kg^{-1} , and for Zn between 10 and 300 mg kg^{-1} (average 40) (Miljøstyrelsen 1998). All measured total HM concentrations at test site 1 and the total concentrations of Cd and Cu in soils from test site 2 are above the background level. Concentrations in soils from test site 3 and from all reference sites are close to or even below the given background levels. In Denmark, two standards (Table S6, Supporting Information) are used to classify soil contamination levels as follows: (1) the soil quality standard, below which the soil can be used for any purpose, and (2) the enclosure standard, where exceeding concentrations indicate that the soil should be remediated or covered to avoid risks (Miljøstyrelsen 2010). Accordingly, test site 1 can be classified as strongly polluted and above enclosure standard for Cd, Ni, and Zn; test site 2 can be classified as slightly polluted with only Cd above the quality standard. Test site 3 is not polluted; all investigated HM are below the quality standard.

Concentrations in tree cores The concentration data in Table 1 together with the Y-intercepts in Table 2 suggest that both willows and poplars show a certain background concentration in wood that is relatively stable at all reference sites and at the

non-polluted test site 3. This typical background concentration in wood is similar for poplars and willows and is about 0.5 mg kg^{-1} for Cd (between 0.5 and 0.9 mg kg^{-1}), $1.6 (1.1\text{--}1.8) \text{ mg kg}^{-1} \text{ dw}$ for Cu, $0.3 (0.1\text{--}0.5) \text{ mg kg}^{-1}$ for Ni, and 20 to 25 mg kg^{-1} for Zn. Other studies show similar background concentrations in poplars and willows (Evangelou et al. 2013; Algreen et al. 2012). Algreen et al. measured concentrations in tree core samples from the former dump site Møringa in Norway. Concentrations of Cd in willows and poplars were similar and ranged from 0.33 to 0.76 mg kg^{-1} wood. This is comparable to the results found in this study, except for Cd in willows from the highly polluted test site 1. The same was seen for Cu, where the range in samples from Møringa (1.95 in reference willows and 1.33 to 1.66 mg kg^{-1} for poplars from reference and test) is close to the Cu contents in both willows and poplars from Danish reference and less polluted sites (average 1.11 to 1.79 mg kg^{-1} , Table 1). The Cu concentration in willows from the Norwegian test site was 3.05 mg kg^{-1} and was clearly elevated, but less than in this study (10.7 mg kg^{-1} in willows from test site 1). Concentrations of Zn in poplars from Norway were 27 to 32.5 mg kg^{-1} (reference and test site), and in willows 15 and 32 mg kg^{-1} (first and second campaign, reference site) and 25 and 36 mg kg^{-1} (test site), which is also close to the results obtained in the present study, again with the exception of Zn in willows from the highly polluted test site 1. Zacchini et al. (2011) studied the cadmium accumulation in roots and leaves from willows and poplars and found higher Cd content in poplar roots than in willow roots, $9,735$ and $4,082 \text{ mg kg}^{-1}$ respectively, and consequently lower Cd content in poplar leaves (23 mg kg^{-1}) than in willow leaves (376 mg kg^{-1}). The difference between Cd in exposed and control willows was higher than in our study. Reimann et al. (2001) and Djingova et al. (2004) studied the content of Cd, Cr, Cu, Ni, and other metals in leaves of willows and poplars. Their results confirm higher HM concentrations in willow (leaves) than in poplar (leaves), except for Cr. The capacity of *Salix* clones for biomass production and accumulation of heavy metals was tested by Mleczek et al. (2010). The maximum differences between the highest and lowest heavy metal content in shoots from the *Salix* clones were for Cd 84 %, Cu 90 %, mercury 167 %, Pb 190 %, and Zn 36 %. In the study of Pietrini et al. (2010), a significant variability for translocation of Cd to woody parts was found among 10 poplar clones. The average content of Cd in the stems of poplar clones grown in the presence of $50 \text{ } \mu\text{M CdSO}_4$ (at the end of the experiment) was 1.56 mg , ranging from 0.75 to 2.22 mg . The standard deviation was at 0.50 mg , and the coefficient of variation (CV) thus 32 % (neither stem mass nor Cd concentration were given). These results show that uptake and concentration of HM in wood vary considerably with species and even with clone. High standard deviation and CV was also found in the previous study, but, nonetheless, a significant difference to the

Fig. 2 Measured concentrations of Cd, Cu, Ni, and Zn in wood (willow and poplar) versus measured total concentrations in soil. Lines represent linear fit (poplar dotted); black arrows indicate usual Danish background in soil (Miljøstyrelsen 1998)



results from the reference site was obtained, despite the low number of sample events (Tables S3 and S4).

Willows (*Salix* sp.) and poplars (*Populus* sp.) both belong to the family of Salicaceae. In Norway (Algreen et al. 2012), most trees were from *Salix caprea* goat willows and *P. nigra* black poplars. At reference sites 1 and 3 and test site 3, *Salix alba* (white willow or silver willow) and at test and reference site 2 *P. nigra* were sampled. At test site 1, *S. viminalis* (basket willows) and balsam poplars (*P. trichocarpa*) were most frequent. It seems that all these trees from the family Salicaceae have a rather constant background concentration of the studied HM in their wood, not varying much with habitat and species. If such a typical background level is confirmed in future studies, elevated concentrations can be detected without reference measurements. This simplifies phytoscreening for HM considerably.

Relation between concentration in wood and in soil The BCF values calculated for each site using Eq. (1), i.e., from the average concentrations, decrease for both species with increasing soil concentration (Table 2). Inspection of Fig. 2 offers some explanation; at low concentrations in soil, HM in wood are at almost constant level, independent of the HM in

soil. Only at rather high concentrations in soil, levels in wood increase, too. This increase is not very well described by the linear fit, i.e., the transfer soil-to-plant is nonlinear with respect to soil concentrations. The regressions provide reasonable predictions only for the highest values. This is in accordance with recent findings by Tuovinen et al. (2011). It can be speculated that below a certain threshold, enzyme systems regulate HM uptake, and above, metals break through. Such pattern has been observed for essential heavy metals (McLaughlin et al. 2011), but also for salt (Trapp et al. 2008). The concentrations of Cu and Zn in poplar wood are rather unrelated to the respective concentrations in soil; the slope is even negative. Interestingly, both metals are known as essential for plant growth (Marschner 1995). The regression with the easily extractable concentration in soil improves the R^2 only in two out of eight cases, indicating that the widely applied CaCl_2 -extractable fraction of HM in soil is not always a good predictor for the bioavailable fraction of HM. Smith et al. (2012) compared the residues of Cu and Zn and their effects in plants with the bioavailable concentration in soil determined by several extraction methods, among them CaCl_2 -extraction. The authors found that effects of Cu on plants best correlated with total concentration.

Table 2 Soil-to-plant transfer (kg/kg dw) of average measured concentrations in wood and total soil per site calculated with Eq. 1; slopes of the linear regression between concentration in wood and in total soil of all sites (bioconcentration factors BCF kg/kg dw); Y-intercepts of regression (background concentration mg/kg dw); coefficient of determination R^2 (in bold: significant at $\alpha=5\%$); number of sample pairs n. Regressions are significant at $R^2 > R^2_{sig} = 0.569$ for willows and 0.264 for poplars

| | | Linear regression | | | | | |
|--------------------------|----|-------------------|-------|--------------------------|----------------------------------------|----------------|----------------|
| | | Slope (BCF) | | | Y-intercept (Background concentration) | | R^2 |
| Test site 1, willow area | Cd | 0.15 | 3.3 | Ref. site 1, willow area | Test site 3, willow area | Willows (n=7) | Willows (n=7) |
| | Cu | 0.015 | 0.35 | Ref. site 1, willow area | Test site 3, willow area | Willows (n=7) | Willows (n=7) |
| | Ni | 0.0035 | 0.020 | Ref. site 1, willow area | Test site 3, willow area | Willows (n=7) | Willows (n=7) |
| | Zn | 0.036 | 0.88 | Ref. site 1, willow area | Test site 3, willow area | Willows (n=7) | Willows (n=7) |
| Test site 1, poplar area | Cd | 0.066 | 1.5 | Ref. site 1, poplar area | Test site 2, poplar area | Poplars (n=15) | Poplars (n=15) |
| | Cu | 0.0017 | 0.53 | Ref. site 1, poplar area | Test site 2, poplar area | Poplars (n=15) | Poplars (n=15) |
| | Ni | 0.0059 | 0.045 | Ref. site 1, poplar area | Test site 2, poplar area | Poplars (n=15) | Poplars (n=15) |
| | Zn | 0.011 | 0.64 | Ref. site 1, poplar area | Test site 2, poplar area | Poplars (n=15) | Poplars (n=15) |

BCF by Eq. 1

Table 3 HM concentrations measured at test site 1 in soil C_{Soil} in 2001 (data from Oberender et al.¹⁶) and 2011 (this study) as well as calculated phytoextracted mass Δm (for 1 m² soil) and calculated change of concentration ΔC_{Soil} , based on measured concentrations in wood C_{Wood}

| | Metal | C_{Soil} 2001 (mg kg ⁻¹) | C_{Soil} 2011 (mg kg ⁻¹) | C_{Wood} site 1 (mg kg ⁻¹) | Growth (kg) | Δm (mg) | ΔC_{Soil} (mg kg ⁻¹) | % of C_{Soil} 2001 ^a |
|-----------------|-------|----------------------------------------|----------------------------------------|------------------------------------------|-------------|-----------------|------------------------------------------|-----------------------------------|
| Area of willows | Cd | 7.7 | 24 | 3.08 | 10 | 30.8 | 0.034 | 0.440 |
| | Cu | 370 | 588 | 10.7 | 10 | 107 | 0.12 | 0.032 |
| | Ni | 89 | 132 | 0.48 | 10 | 4.8 | 0.0053 | 0.006 |
| Area of poplars | Zn | 1,800 | 3,270 | 106 | 10 | 1,060 | 1.16 | 0.065 |
| | Cd | 11 | 11 | 0.62 | 15 | 9.3 | 0.010 | 0.093 |
| | Cu | 717 | 651 | 1.21 | 15 | 18.2 | 0.020 | 0.003 |
| | Ni | 170 | 153 | 0.74 | 15 | 11.1 | 0.012 | 0.007 |
| | Zn | 3467 | 2,964 | 32.9 | 15 | 494 | 0.54 | 0.016 |

^a Percentage of reduction due to phytoextraction

Mass balance calculations Test site 1 had been established as a test site for phytoremediation (cf. section test sites) in 1999. Table 3 compares the measured concentrations of HM in soil in 2001 (Oberender et al. 2009) and in 2011 (this study, from Table 1). The removal of HM from soil into wood by phytoextraction was calculated with Eqs. (2) and (3), based on measured concentrations in wood (also shown in Table 3). The extraction with willows is for Cd, Cu, Ni, and Zn much higher (factors 5 to 8) than with poplars, despite the lower biomass production. The highest relative extraction (percent of 2001 soil concentration) is calculated for Cd and willows, but still less than 1 % in 10 years. The extraction by poplars is below 1‰ for all four HM. The measured concentrations in 2011 are sometimes below, sometimes above, than those from 2001. There were small differences in the sampling method (depth in 2001, 0.85 to 1.25 m; in 2011, 0.7 m) and extraction procedure (aqua regia in 2001, HNO_3 in 2011). Due to the large variance of the HM analysis in soil, a real difference between the HM concentrations in soil determined in 2001 and 2011 cannot be seen.

The time it takes to reach legal standards in soil by phytoextraction alone was calculated with Eq (5). The slowest metal extraction is for Ni, where the measured concentration $C(0)$ on the poplar area is 170 mg kg^{-1} , the quality standard $C(t)$ is 30 mg kg^{-1} , and BCF poplar is $0.0059 \text{ kg kg}^{-1}$. With a ΔM_{Wood} of $1.5 \text{ kg m}^{-2} \text{ year}^{-1}$ for poplar, and a M_{Soil} of 910 kg as above, k is $9.73 \times 10^{-6} \text{ year}^{-1}$, and t is 178,360 years. Also with the more optimistic BCF from the reference site (0.045 kg kg^{-1}), the extraction down to the standard still takes more than 20,000 years. Both numbers are not acceptable for an applied phytoremediation project. The calculated extraction efficiency is higher for the other sites, both because absolute concentrations in soil are lower, and relative bioconcentration factors (Table 2) are higher. However, none of the other sites is above the enclosure level that was set by the Danish EPA (Miljøstyrelsen 2010), and thus, no treatment is required. Trees on test site 1 have in 2013 partly been cut down, and the site is now used as parking place, perhaps also due to the slow extraction. Growing trees still is a reasonable way of using such highly polluted sites. Benefits of a plant cover are reduction of leaching and erosion (Hammer et al. 2003; Trapp and Karlson 2001). Moreover, forests provide a habitat for wildlife, relaxation for humans, fixing of CO_2 , and can be harvested.

Hammer et al. (2003) investigated the phytoextraction of Cd and Zn with willow (*S. viminalis*) in field trials in Switzerland. Initial total concentrations in soil were lower than in our study, namely 2.3 mg kg^{-1} Cd and 650 mg kg^{-1} Zn. Concentrations of Cd and Zn in shoots (leaves and wood) decreased over the 5-year period from 3.6 to 1.5 mg kg^{-1} Cd and from 200 to 70 mg kg^{-1} Zn. Total extraction increased due to an increase of biomass production, and was at maximum about 60 g Cd ha^{-1} (6 mg m^{-2}) per year and about 5 kg Zn ha^{-1} (500 mg m^{-2})

per year, which is two times more for Cd and five times more for Zn than in our study (Table 3).

Conclusions

Tree cores from willows and poplars from a strongly polluted, a moderately polluted, and a practically non-polluted test site and from reference sites were sampled and analyzed for heavy metals (HM). Concentrations of Cd, Cu, Ni, and Zn in willow wood sampled from the highly polluted site were significantly elevated, compared to references, indicating the feasibility of phytoscreening for some HM with willows at strongly polluted sites. Differences were less clear for poplar. Background concentrations in wood from less and non-polluted sites were in a relatively narrow range and varied little with species, sampling time, and other conditions.

Extraction efficiencies of HM from the highest polluted site were best with willows due to the higher concentrations in wood and despite the lower biomass, but were still below 0.5 % over 10 years for willows and Cd, and below 1‰ for the other HM and for poplars. Nonetheless, we do recommend to plant trees on HM-contaminated sites due to several beneficial effects.

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